*HISTOCOMPATIBILITY GENETICS AND IMMUNOSUPPRESSION**

FRITZ H. BACH†

DEPARTMENT OF MEDICAL GENETICS, UNIVERSITY OF WISCONSIN SCHOOL OF MEDICINE

Professor Cournand has asked me to review very briefly two areas of transplantation biology: first, the genetic basis of histocompatibility in man, including pairing of donor and recipient for transplantation; and second, the use of immunosuppressive drugs to inhibit the cellular processes which lead to homograft rejection. I must emphasize at the outset that I have worked only in the first of these areas and will base my comments about immunosuppression solely on the work of others.

Dr. Billingham has discussed the genetic basis of histocompatibility in the mouse and rat. In those species there is one genetic system (locus), called the major histocompatibility locus, which controls the strong histocompatibility antigens which if present on donor tissue and absent from the host will lead to rapid rejection of the donor tissue. There are many other independent loci which determine antigens, incompatibility for which will lead to graft rejection; however, immunosuppression is more effective and tolerance more easily obtained if the incompatibilities present are determined only by these minor loci.

In man two independent genetic systems are of great importance in determining graft survival; several "minor" systems no doubt exist. First, incompatibility for the ABO blood group system is generally regarded as an absolute contraindication to transplantation; second, incompatibility for the major histocompatibility locus in man (HL-A), which is perhaps analogous to the major loci in mouse and rat, can represent a very strong barrier, at least to skin transplantation. Since it is relatively simple to pair donor and recipient for the ABO locus, because of the limited polymorphism, and much is known about that locus, I will limit my comments to the HL-A system.

There are two approaches to determining donor-recipient compatibility at HL-A. "Typing" uses isoimmune antisera obtained from multiparous females, who have made antibodies to fetal antigens "inherited" from the father and not possessed by the mother, or from specifically immunized individuals. The majority of available antisera identifies antigens controlled by the HL-A system. Pairing on this basis attempts to minimize antigenic incompatibility. Donor-recipient pairing can alternatively be achieved by "matching" tests, such as the mixed leukocyte culture test. In this test the response of recipient lymphocytes to the HL-A antigens present on the leukocytes of the donor is measured *in vitro*. The recipient's cells respond by enlarging, synthesizing DNA, and dividing; it is possible to assay a one-way reaction by treating the stimulating cells of the donor with mitomycin-C, thereby inhibiting DNA synthesis in those cells. With the mixed leukocyte culture test, the degree of response (radioactive thymidine incorporation into DNA by the untreated responding cells of the potential recipient) is a meaningful measure of the degree of HL-A antigenic incompatibility as correlated with the number of HL-A alleles by which two members of a family differ. No definition is given of the specific antigens responsible for the incom-

patibility. Cells of siblings who are identical at HL-A do not stimulate each other in these tests. For both typing and matching it must be assumed that the antigenic phenotype on the peripheral blood lymphocytes, usually used as test cells, adequately reflects that on the kidney, liver or other tissue.

The HL-A system is complex in two regards: there are many different HL-A alleles in the population (minimum estimates suggest 20–30 alleles), and each allele may have associated with it more than one antigen. Most individuals are heterozygous at HL-A due to the high degree of polymorphism; virtually all unrelated individuals differ from each other by two HL-A alleles. Six HL-A antigens (named HL-A 1, 2, 3, 5, 7, and 8) have been sufficiently well defined serologically to allow international agreement on the above nomenclature. However, other HL-A antigens do exist and can be detected in several laboratories; still others may not yet be defined. Whether all the "important" antigens have been defined ("important" in that they will lead to graft rejection despite immunosuppressive therapy if incompatibility for the antigen exists) is also not clear. Thus while typing results are significantly predictive of graft survival in siblings (in whom a simplified situation obtains and pairing by typing tests need only assure identity of inheritance of parental HL-A alleles by identifying which paternal and which maternal allele each sibling has inherited), they are less clearly correlated with graft survival in unrelated pairs (in whom it is essential to rule out antigenic incompatibility). At the present time the failure to achieve significant prolongation of graft survival in individuals who "type identically" should probably be ascribed to undetected antigenic incompatibility. Since typing antisera does measure some HL-A antigens, pairing for identity for recognized antigens will lead to a statistical improvement in transplant survival—the degree of improvement being dependent on the effective percentage of antigens defined; in any one case, however, the problem of undefined antigens remains. Further, in cases where there is some antigenic incompatibility, we presently have no knowledge about incompatibility "strength" of the various antigens. Despite the above potential problems, it is clear that typing, which can be accomplished in a few hours, should be done to achieve the best pairing possible.

Mixed leukocyte culture tests take several days to perform; the shortest method we currently use gives results within three days. For organ transplants when this length of time is available (living donor and rare cadaveric donors), the test offers information in addition to that obtained by typing. For reasons outlined above, phenotypic identity by typing is no assurance of HL-A identity; mixed culture test results may give a more reliable measure of HL-A identity as well as providing meaningful quantitative information about the degree of disparity at HL-A. Even if the mixed leukocyte culture tests cannot be used prospectively for cadaver transplantation, retrospective information will add knowledge essential to more effective donor-recipient pairing in man.

The major questions confronting us at present are: how much incompatibility at HL-A can be tolerated and still permit good transplant survival (if indeed such a simple relationship exists), and how often will we find an unrelated donor who fits into this "acceptable" incompatibility range—which will determine how much regional sharing of organs will be needed. I think that a combined use of

typing and matching will give us these answers in a relatively short period of time. At present, however, one must be careful to distinguish between genetic identity at HL-A as exists in siblings inheriting the same parental chromosomes, effective identity as may be defined by mixed leukocyte culture tests, serotypic identity as defined by a limited number of antisera, and identity for the important antigens—a concept of purely theoretical nature at the present time.

The use of immunosuppressive agents in homotransplantation has been one of the major factors allowing the progress made in this field. While at the present time in man it appears that graft survival, using immunosuppression, is more successful in the well-paired donor-recipient combinations, and while there is even the question, raised by experiments in the rat, whether siblings who are identical at HL-A need immunosuppression, many investigators feel that further advances and refinements of presently available immunosuppressants will permit transplantation across very strong histocompatibility differences.

Immumosuppressive drugs can be divided into two categories. General immunosuppressive agents such as steroid, azathioprine, actinomycin C, etc., inhibit cellular metabolism not only in cells involved in delayed type immunological reactions, which are probably the principal cause of homograft rejection, but also in cells mediating immediate type, or antibody forming, immunological reactions which are important for resistance to many forms of infection. Specific agents such as antilymphocyte serum and antilymphocyte globulin, while inhibiting delayed type reactions are less suppressive of the systems responsible for fighting many forms of infection. Antilymphocyte serum can be markedly immunosuppressive—in some cases prolonging survival of heterografts (grafts from members of another species) which are normally rapidly and violently rejected. Some investigators feel the drug has been of little therapeutic value in man while others are impressed with its potential. One would expect that antilymphocyte globulin is, or will be if used in therapeutic doses, a very potent immunosuppressive agent in man. Immunosuppressive regimens use a combination of steroids and azathoprine (imuran) in most cases, with or without antilymphocyte serum. Whereas initially fairly high doses are given, the dosage is rapidly tapered after transplantation to low level maintenance doses. Drug therapy is continued indefinitely. In the event of a rejection crisis, the patient may be temporarily given increased doses of steroids or supplemental therapy with a drug such as actinomycin C.

Long-term immunosuppressive drug therapy is not without its attendant dangers. The increased incidence of infection in these patients is well recognized. Another, more recently recognized, complication appears to be an increased incidence of neoplasms in the suppressed patients. Of the 2500 or so transplants done, 15 have developed malignancy—a number significantly higher than expected for this age group. This finding fits in well with the concept that a competent immunological system is essential to prevent the proliferation of newly arising neoplastic cells. The increased incidence of tumors in immunosuppressed animals and in children with immunological deficiency diseases further supports this concept.

Certainly the goal of many transplantation biologists is to induce in the recip-

ient specific immunological tolerance of donor tissue instead of administering prolonged immunosuppression. There are data which suggest that some kidney recipients may have become "tolerant" of their donor tissue; however, at present we do not understand the reason for the state of specific non-reactivity in these cases. In the future, perhaps tolerance will be intentionally induced by the administration of specific soluble transplantation antigens with or without short term immunosuppression or by bone marrow transplantation from the organ donor. The tolerant state would allow acceptance of the donor organ without impairing the remaining immunological reactivity against other antigens—leaving intact the recipient's capabilities of immune response to foreign antigens on bacteria, viruses, tumor cells, or other agents.

* This is paper #1315 from the Laboratory of Genetics, University of Wisconsin, Madison.
† Established Investigator of the American Heart Association.